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Relationship between origin and nutrient content of Croatian common bean landraces

Poveznica porijekla i količine hranivih tvari hrvatskih tradicijskih kultivara graha

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Abstract

Common bean (*Phaseolus vulgaris* L.) is an important legume crop. In Croatia, many different common bean landraces are grown mostly on small farms and in kitchen gardens. The aim of this research was to determine and compare nutrient content of Croatian common bean landraces and to test the existence of the relationship between common bean nutrient content with origin of accessions. A total of 226 common bean accessions from all over Croatia were collected, grown in unreplicated field trial at the experimental field in Zagreb, harvested and analyzed for nutrient content. Results showed high nutrient content, but clear difference between Mesoamerican and Andean gene pool regards the nutrient profile of Croatian common bean landraces was not determined. Regardless of gene pool, the average nutrient content in dry weight of Croatian common bean landraces was in range: 3.13–3.92% N, 0.49–0.57% P, 1.38–1.66% K, 0.34–0.42% Ca, 0.17–0.2% Mg, 63.75–75.77 mg Fe·kg^{-1}, 13.55–19.94 mg Mn·kg^{-1} and 24.98–29.32 mg Zn·kg^{-1}. Present research provides a solid basis for future breeding programs and improvement of common bean traits.

Keywords: macronutrients, micronutrients, minerals, phaseolin type, *Phaseolus vulgaris* L.

Sažetak

Grah (*Phaseolus vulgaris* L.) važna je kultura iz porodice mahunarki. U Hrvatskoj, različiti tradicijski kultivari graha pretežito se uzgajaju na malim gospodarstvima i u povrtnjacima. Cilj ovog istraživanja bio je utvrditi i usporediti količinu hranivih tvari u hrvatskim tradicijskim kultivarima graha, te utvrditi postojanje povezanosti količine hranivih tvari i podrijetla primki. Ukupno je prikupljeno 226 primki graha, iz cijele Hrvatske, koje su uzgojene u poljskom pokusu bez ponavljanja na pokušalištu u Zagrebu. Nakon berbe izvršena je analiza na količinu hranivih tvari. Rezultati su pokazali značajnu količinu hranivih tvari, ali jasne razlike, temeljem određenih količina hraniva u hrvatskim tradicijskim kultivarima graha, nisu utvrđene između kultivara mezoameričkog i andskog porijekla. Neovisno o porijeklu kultivara, prosječna količina hranivih tvari u suhoj tvari hrvatskih tradicijskih kultivara graha bila je u sljedećim rasponima: 3,13–3,92% N, 0,49–0,57% P, 1,38–1,66% K, 0,34–0,42% Ca, 0,17–0,2% Mg, 63,75–75,77 mg Fe·kg^{-1}, 13,55–19,94 mg Mn·kg^{-1} i 24,98–29,32 mg Zn·kg^{-1}. Provedeno istraživanje predstavlja dobar temelj za buduća istraživanja s ciljem oplemenjivanja i poboljšanja svojstava graha.

Ključne riječi: fazeolin tip, makrohraniva, mikrohraniva, minerali, *Phaseolus vulgaris* L.

Introduction

Common bean (*Phaseolus vulgaris* L.) is a major legume crop and the most important grain legume for direct human consumption (Broughton et al., 2003) since it makes up to 50% of the worldwide grain legume consumption (Talukder et al., 2010). It has high nutritional value with substantial protein, mineral, vitamin, and fiber concentrations (Campos-Vega et al., 2013). Furthermore, it is an important source of calcium, iron, magnesium, phosphorus, potassium, sodium, and zinc (Camara et al., 2013). Common bean zinc content is one of the highest among vegetable sources (Beebe et al., 2000) and it can be a potential solution for zinc deficiency, which has been recognized as a global public health problem (Guzman-Maldonado and Peredes-Lopez, 1998).

Common bean was domesticated twice independently, in Mesoamerica and in the Andes, and these two independent events gave origin to two major domesticated gene pools (Angioi et al., 2009). Ribeiro et al. (2012) reported that genotypes of Mesoamerican origin have superior seed mineral content compared to genotypes of Andean origin. Thus, common bean is known for its morphological variability and adaptability to different environments, creating a wide range of local varieties or landraces, as well as different technological quality of seeds (Perina et al., 2014). Moreover, studies carried out using phaseolin type as a discriminator showed that both gene pools are present in Europe, with a higher frequency of the Andean type (Bellucci et al., 2014; Maras et al., 2015). In many European countries, the selective pressure operated by farmers over time on the common bean genetic pool of introduced accessions led to the creation of well adapted landraces (Piergiovanni and Lioi, 2010). Carović-Stanko et al. (2017) reported that common bean landraces are an important source of genetic resistance for plant breeders, as they often

contain alleles for local adaptations, disease resistance, tolerance to climatic adversities etc. According to Vasić et al. (2009) and Savić et al. (2014), the use of common bean landraces seems to be of a promising genetic potential, either for variety release, or for plant breeding programs. Furthermore, common bean landraces presented a better nutritional performance by having similar (Bevilaqua and Antunes, 2015) or higher (Gouveia et al., 2014) average seed mineral content, compared to commercial cultivars.

The aim of the present research was to determine and compare nutrient content of common bean landraces collected from all over Croatia and grown under equal conditions and test the existence of the relationship between common bean nutrient content with origin of accessions.

Materials and methods

Field trial – soil and plant material

Research was carried out during growing season 2014 on 226 common bean accessions. Seed samples of the studied accessions were collected from all over Croatia (Figure 1). Accessions were grown under equal conditions in unreplicated field trial on the experimental field of the Department of Seed Science and Technology, Faculty of Agriculture, University of Zagreb, Croatia (lat. 45º 49' N; long. 16º 20' E). Physical and chemical properties of the soil used in the research are shown in Table 1. The utilized bean growing technology followed the normal working procedure for bean production. Harvested common bean seeds from each field plot were collected and homogenized samples were created.

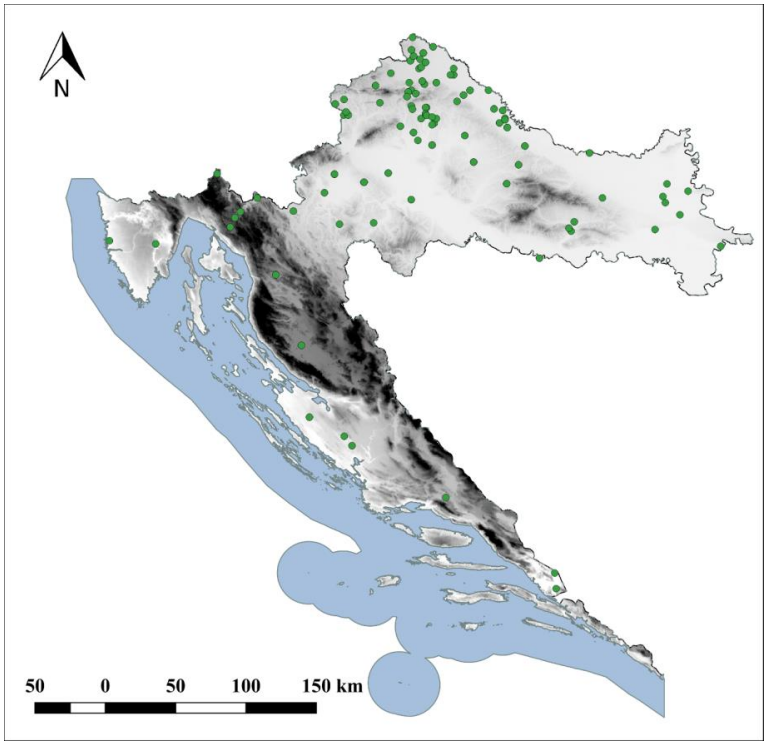

Figure 1. Sampling locations for common bean in Croatia

Table 1. Physical and chemical properties of the soil from experimental field

Sand[z]	Silt[z]	Clay[z]	pH[y]		C_{org}[x]	N[w]	P_2O_5[v]	K_2O[v]
	%		H_2O	KCl	%		mg/100 g	
17.2	67.4	15.4	6.31	5.09	0.89	0.09	11.7	16.2

[z]Soil particle size distribution was determined by pipette-method with sieving and sedimentation (HRN ISO 11277:2011); [y]pH potentiometrically (HRN ISO 10390:2005); [x]Organic carbon content (C_{org}) determination after dry combustion (HRN ISO 11277:2004); [w]Total nitrogen by modified Kjeldahl method (HRN ISO 10694:2004); [v]Phosphorus and potassium by ammonium lactate method in accordance with Egner-Riehm-Domingo (Egner et al., 1960).

Accessions were classified according to morphological traits using the descriptor list for common bean used in *Phaseolus* Database (http://www. genbank.at/en/ecpgr-phaseolus.html), as reported earlier by Vidak et al. (2015). Classes represent the most widespread landraces in Croatia, named: 'Biser', 'Tetovac', 'Kukuruzar', 'Visoki Trešnjevac – V. Trešnjevac', 'Niski Trešnjevac – N. Trešnjevac', 'Zelenčec' and 'Puter'. According to the methodology proposed by Amurrio et al. (2000) and De la Cuadra et al. (2001), the researched morphotypes match market classes in the following order: 'Biser' = Navy Bean; 'Tetovac' = White Kidney Bean; 'Kukuruzar' = Light Brown Kidney Bean; 'V. Trešnjevac' = Cranberry Bean; 'N. Trešnjevac' = Cranberry Bean; 'Zelenčec' = Green Kidney Bean; 'Puter' = Beige Kidney Bean.

According to Genchev and Kiryakov (2005) and based on the accessions' growth habit, morphotypes were classified in two groups: determinate – dwarf bean ('Biser', 'N. Trešnjevac', 'Zelenčec' and 'Puter') and indeterminate – climbing bean ('Kukuruzar', 'V. Trešnjevac' and 'Tetovac').

Nutrient content analyses

Seed samples (dried at 70 °C) were divided into three subsamples and analyzed. The results are presented as sample averages ± SD. Nitrogen was determined using the Kjeldahl method (AOAC, 2015). Seed crude protein content was calculated by the multiplication of average nitrogen content with the factor 6.25 (AOAC, 2015). After digestion with concentrated HNO_3 (MILESTONE 1200 Mega Microwave Digester), phosphorus content was determined using a spectrophotometer, potassium by a flame photometer, while calcium, magnesium, iron, zinc and manganese were analyzed by an atomic absorption spectrophotometer (AAS) (AOAC, 2015).

Phaseolin type determination

The data on phaseolin type for all analyzed accessions were overtaken from the research reported by Carović-Stanko et al. (2017).

Statistical data analyses

Mineral content data of common bean accessions were subjected to statistical analyses to test the differences between seven morphotypes representing the most widespread landraces in Croatia. Applied statistical methods included simple one-way analysis of variance and multiple comparisons, using base R (R Core Team, 2014) and "multcomp" package (Hothorn et al., 2008), respectively.

Results and discussion

Phaseolin type analysis showed that 75 accessions (corresponding to landraces 'Biser', 'Tetovac' and 'Kukuruzar') had phaseolin type I ("S") predominant in the Mesoamerican gene pool, whereas the rest of landraces ('N. Trešnjevac', 'V. Trešnjevac', 'Zelenčec' and 'Puter') belong to Andean gene pool characterized by the phaseolin type II ("H" or "C") or III ("T") (Table 2). Similar findings were reported by Šustar-Vozlič et al. (2006) for Slovenian common bean landraces were phaseolin type "C" and "T" were characteristic for landraces from two sub-groups of Andean gene pool, whereas "S" type predominated in Mesoamerican group. In addition, results are in accordance with Bellucci et al. (2014) who reported that higher number of common bean accessions in Europe belong to Andean gene pool. Furthermore, Maras et al. (2015) reported a strong prevalence of accessions of Andean origin over Mesoamerican in the Mediterranean countries (Bosnia and Herzegovina – 60%, Croatia – 67%, Serbia – 63%, and Slovenia – 67%), while Macedonia resembles more to the countries on the east with predominantly Mesoamerican genotypes.

Table 2. Growth habit and phaseolin type of accessions used in research

Landrace (morphotype)	NoAcc	GrHab	PhaType
'Biser'	11	D	I
'Tetovac'	25	I	I
'Kukuruzar'	39	I	I
'Visoki Trešnjevac'	29	I	II
'Niski Trešnjevac'	78	D	III
'Zelenčec'	34	D	III
'Puter'	10	D	III
Total	226		

NoAcc – number of accessions; GrHab – growth habit: PhaType – phaseolin type; D – determinate growth habit; I – indeterminate growth habit; I – Andean origin; II, III – Mesoamerican origin.

Analysis of variance for mineral nutrients content detected significant differences between morphotypes in N, P, K, Fe, Zn and Mn content (Table 3).

Table 3. P values from the analysis of variance for mineral nutrients content

Source of variability	N	P	K	Ca	Mg	Fe	Zn	Mn
Morphotype	<0.001	<0.001	<0.001	0.572	0.082	<0.001	<0.001	<0.001

Nitrogen, phosphorus, potassium, calcium and magnesium content of the researched common bean accessions expressed on dry-weight basis are presented in Table 4.

Table 4. Macronutrient content of Croatian common bean landraces

Landraces (morphotypes)	n	Macronutrient content (% DW)[z]				
		N	P	K	Ca	Mg
'Biser'	11	3.15±0.18^{c}	0.57±0.08^{ab}	1.46±0.1^{ab}	0.42±0.07	0.2±0.02
'Tetovac'	25	3.59±0.28^{b}	0.53±0.06^{ac}	1.5±0.08^{a}	0.36±0.13	0.19±0.04
'Kukuruzar'	39	3.69±0.25^{b}	0.55±0.06^{a}	1.46±0.14^{ab}	0.34±0.14	0.18±0.02
'V. Trešnjevac'	29	3.92±0.31^{a}	0.54±0.04^{ac}	1.43±0.1^{ab}	0.35±0.12	0.18±0.02
'N. Trešnjevac'	78	3.2±0.23^{c}	0.52±0.06^{bc}	1.42±0.11^{b}	0.4±0.24	0.19±0.03
'Zelenčec'	34	3.13±0.2^{c}	0.5±0.06^{c}	1.38±0.42^{c}	0.36±0.09	0.18±0.02
'Puter'	10	3.26±0.23^{c}	0.49±0.05^{c}	1.66±0.76^{abc}	0.36±0.11	0.17±0.02
Average value		3.41±0.38	0.53±0.06	1.44±0.25	0.37±0.17	0.18±0.03

[z]Average values of macronutrient content expressed as percentage of dry weight of subsamples ± SD. Different letters within the same column indicate significant differences, with error P≤0.05.

Average nitrogen (N) content in seed dry weight (DW) was 3.41% N DW ± 0.38 SD and was lowest in 'Zelenčec' (3.13% N DW) and highest in V. Trešnjevac' (3.92% N DW). Average nitrogen content of climber common beans ('Tetovac'; 'Kukuruzar'; and 'V. Trešnjevac') was significantly higher compared to dwarf common beans ('Biser', 'N. Trešnjevac', 'Zelenčec' and 'Puter'). Obtained total nitrogen values were similar to or slightly higher compared to data reported by Bevilaqua and Antunes (2015), Barros and Prudencio (2016) and Silveira et al. (2016) (3.3, 3.78, and 2.9–

3.5% N DW, respectively). Whereas, Moraghan and Grafton (2001) and Gouveia et al. (2014) reported higher values compared to results of present research (4.2 and 4.83% N DW, respectively). As common bean is a legume crop, high N content is important for its high protein content (Petek et al., 2012). Average crude protein content in DW of common bean seeds was 21.31% which is similar to results reported by Shimelis and Rakshit (2005) and de Almeida Costa et al. (2006).

Phosphorus (P) values varied from 0.49% P DW ('Puter') to 0.57% P DW ('Biser'), whereas the average phosphorus content for all landraces was 0.53% P DW ± 0.06 SD. In landraces of Andean gene pool ('Puter', 'Zelenčec', 'V. Trešnjevac' and 'N. Trešnjevac') determined P content was significantly lower compared to landraces of Mesoamerican gene pool ('Tetovac', 'Kukuruzar' and 'Biser'). Phosphorus content values in dry bean seeds reported by Gouveia et al. (2014) and Bevilaqua and Antunes (2015) (0.63 and 0.75% P DW, respectively) were higher compared to present results, whereas other authors reported lower values (Beebe et al., 2000; Barros and Prudencio, 2016; Silveira et al., 2016). Phosphorus is an important mineral for plant metabolism and in seeds is present as constituent of nucleic (Bergman, 1992) and phytic acid (Guzman-Maldonado et al., 2000).

Average potassium (K) seed content across all examined common bean landraces was 1.44% K DW ± 0.25 SD. Significantly lower seed potassium content, compared to the other landraces, was determined in 'N. Trešnjevac' (1.42% K DW) and 'Zelenčec' (1.38% K DW), both from Andean gene pool. Gouveia et al. (2014) reported higher content of potassium in common bean seed, up to 2.49% K DW. Other authors (Bevilaqua and Antunes, 2015; Barros and Prudencio, 2016; Silveira et al., 2016) reported similar or lower values compared to K content determined in the present research.

Determined common bean seed calcium (Ca) content in all studied landraces varied from 0.34 ('Kukuruzar') to 0.42% Ca DW ('Biser'), with no significant differences among the researched landraces. The average calcium content across examined landraces was 0.37% Ca DW ± 0.17 SD. All determined seed calcium content values were higher compared to previously reported data where calcium content ranged from 0.09 to 0.19% Ca DW (Beebe et al., 2000; Guzman-Maldonado et al., 2000; Barros and Prudencio, 2016), or 0.3% Ca DW reported by Moraghan and Grafton (1997). Higher calcium and lower potassium content found in the present research could be explained by their known antagonism in plant uptake (Marschner, 2011).

There were no significant differences in magnesium (Mg) content among examined Croatian common bean landraces. Magnesium content varied from 0.17 to 0.2% DW, with the average of 0.18% Mg DW ± 0.03 SD. All reported data compared on dry-weight basis were higher or similar to values reported by Moraghan and Grafton (1997; 2001), Gouveira et al. (2014) and Silveira et al. (2016).

Common bean landraces micronutrients content (iron, manganese and zinc) on dry-weight basis are shown in Table 5.

The highest iron (Fe) content was found in landrace 'N. Trešnjevac' from Andean gene pool, while the lowest in 'Kukuruzar' from Mesoamerican gene pool (Table 5). Average seed iron content was 71.83 mg $Fe \cdot kg^{-1}$ DW ± 10.74 SD. Iron content determined in Croatian common bean landraces is higher compared to data reported by Talukder et al. (2010), Kato et al. (2015) and Morais et al. (2016). However, higher

values were reported by Guzman-Maldonado et al. (2000) (109 mg Fe·kg^{-1} DW) and Shimelis and Rakshit (2005) (84 mg Fe·kg^{-1} DW).

Common bean origin did not affect the manganese (Mn) content. However, compared to other studied nutrients Mn content showed the greatest variability. The difference between the lowest value of 'Kukuruzar' (15.19 mg Mn·kg^{-1} DW) and the highest manganese content of 'Biser' landrace (19.94 mg Mn·kg^{-1} DW) was 32%. Both mentioned landraces are from Andean gene pool which indicates high variability in Mn content among landraces of the same gene pool. Determined values were similar or higher to those reported by other authors (Bevilaqua and Antunes, 2015; Barros and Prudencio, 2016; Silveira et al., 2016), except Moraghan and Grafton (2001) who reported 16-27 mg Mn·kg^{-1} DW.

The highest zinc (Zn) content was determined in Andean 'V. Trešnjevac' landrace (27.84 mg Zn·kg^{-1} DW), whereas the lowest was determined in the Mesoamerican 'Kukuruzar' landrace (24.98 mg Zn·kg^{-1} DW). Determined values were similar to data reported by Shimelis and Rakshit (2005), Bevilaqua and Antunes (2015), and Kato et al. (2015). Talukder et al. (2010) and Morais et al. (2016) reported almost two times higher values (50 mg Zn·kg^{-1} DW) compared to values obtained in this research.

Table 5. Micronutrient content of Croatian common bean landraces

Landraces (morphotypes)	n	Micronutrient content (mg·kg^{-1} DW)^{z}		
		Fe	Mn	Zn
'Biser'	11	71.81±11.13^{ac}	19.94±2.18^{a}	29.32±3.72^{ab}
'Tetovac'	25	67.39±10.97^{bc}	16.71±2.13^{bd}	25.53±3.63^{bc}
'Kukuruzar'	39	67.33±13.49^{c}	15.19±2.26^{c}	24.98±3.63^{c}
'V. Trešnjevac'	29	73.24±8.89^{ab}	13.55±1.55^{e}	27.84±4.44^{ab}
'N. Trešnjevac'	78	75.77±8.39^{a}	16.45±1.77^{d}	28.93±4.26^{a}
'Zelenčec'	34	72.43±10.65^{ac}	17.86±1.94^{b}	25.85±3.24^{bc}
'Puter'	10	63.75±5.92^{bc}	15.08±1.65^{cde}	26.67±3.13^{ac}
Average value		71.83±10.74	16.21±2.42	27.19±4.2

^{z}Average values of micronutrient content expressed as mg·kg^{-1} of dry weight of subsamples ± SD. Different letters within the same column indicate significant differences, with error P≤0.05.

In this preliminary research of Croatian common bean landraces, seed mineral nutrient content showed differences for all analyzed nutrients, enabling the selection of superior landraces for high seed nutrients content. Higher nutrient content compared to average values of all researched landraces for six (P, K, Ca, Mg, Zn

and Mn) out of eight studied nutrients was shown for dwarf landrace 'Biser' from Mesoamerican gene pool followed by dwarf bean 'N. Trešnjevac' from Andean gene pool. However, the highest value for N was shown for climber bean 'V. Trešnjevac' from Andean gene pool.

Conclusions

In this research on Croatian common bean landraces a great variability of nutritional profile was determined. Seed N content was affected by growth habit where higher N content was determined in dwarf landraces compared to climbing landraces. Origin had significant effect on P content with higher P content determined in landraces from Mesoamerican gene pool. In addition, high potassium content was determined in all three landraces from Mesoamerican gene pool, but the highest was determined in 'Puter' form Andean gene pool. Ca and Mg content were not affected by gene pool or by growth habit. Seed Fe and Zn content was affected by origin where the highest value was recorded for an Andean landrace whereas manganese content showed the greatest variability.

Landrace 'Biser' from Mesoamerican gene pool showed the highest seed mineral content of phosphorus, calcium, magnesium, manganese and zinc. Landraces 'Visoki Trešnjevac', 'Niski Trešnjevac' and 'Puter' from Andean gene pool showed the highest nitrogen, iron and potassium seed content, respectively. The reported results provide a good basis for future breeding and improvement of common bean characteristics, especially regarding nutrient content.

Acknowledgements

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